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Maternal Gametophytic *baseless1* Is Required for Development of the Central Cell and Early Endosperm Patterning in Maize (*Zea mays*)

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ABSTRACT

In angiosperms, double fertilization of an egg cell and a central cell with two sperm cells results in the formation of a seed containing a diploid embryo and a triploid endosperm. The extent to which the embryo sac controls postfertilization events in the seed is unknown. The novel gametophytic maternal-effect maize mutation, *baseless1* (*bsl1*) affects central cell development within the embryo sac, frequently by altering the position of the two polar nuclei. Despite this irregularity, fertilization is as efficient as in wild type. The spatial expression of basal endosperm-specific transcripts is altered in free-nuclear and cellular mutant endosperms. At later stages of seed development, *bsl1* predominantly affects development of the basal endosperm transfer layer (BETL). When *bsl1*/+ diploid plants were pollinated by wild-type tetraploid plants, the BETL abnormalities observed in *bsl1/bsl1*/+/+ tetraploid endosperms were diverse and of variable severity. Moreover, the frequency of kernels with severely perturbed BETL development correlated with the percentage of severely affected *bsl1* central cells. Therefore, BSL1 is likely required in the central cell before fertilization for correct BETL patterning to occur. These findings provide new genetic evidence that a maternal gametophytic component is necessary for correct endosperm patterning.

MOST flowering plants are sexually dimorphic, in that they possess both male and female reproductive organs. The male gametophyte (pollen) typically contains a vegetative nucleus, which contributes to pollen tube growth, and two male gametes (sperm cells) that participate in fertilization. The female gametophyte is usually of the Polygonum type, consisting of two synergid cells, some antipodal cells, and two female gametes—the egg cell and the larger central cell, which contains two polar nuclei (Drews and Yadegari 2002; Yadegari and Drews 2004).

The process of double fertilization is unique to the flowering plants and results in formation of the seed. For this to occur, the pollen tube must first grow toward the ovule, where it is guided to the micropyle and enters the female gametophyte through one of the two synergids. Recent studies have shown that pollen tube growth and guidance are largely controlled by the female gametophyte and in particular by the synergids (Higashiyama *et al.* 2001; Huck *et al.* 2003; Rotman *et al.* 2003; Marton *et al.* 2005). Under wild-type conditions, the pollen tube penetrates the female gametophyte through a degenerated synergid, after which the pollen tube tip ruptures, releasing the two sperm cells. The sperm cells subsequently fuse individually with the egg and central cell to typically form a diploid (1 maternal:1 paternal) embryo and a triploid (2 maternal:1 paternal) endosperm, respectively. Although both seed components are typically identical genetically, their developmental fates diverge significantly. While the life cycle of the embryo is extended to the next generation—where it forms the mature plant—the endosperm life cycle is confined to the seed stage, where it provides nutritional and structural support to the growing embryo (Walbot and Evans 2003; Costa *et al.* 2004).

In many flowering plants, the endosperm undergoes a nuclear-type mode of development (Olsen 2004). Strikingly similar to early Drosophila embryogenesis, the fertilized proendosperm triploid nucleus divides through a series of mitoses that are uncoupled to cell wall formation, to form a free-nuclear structure or syncytium with a single layer of cortical nuclei suspended in the peripheral cytoplasm (Olsen 2001). Following syncytial development, cellularization occurs and various cell types eventually differentiate within the fully cellular endosperm (Costa *et al.* 2004; Olsen 2004).

To date, relatively little is known about the mechanisms regulating early development of the endosperm. On the basis of current genetic evidence, endosperm patterning occurs in two main phases (Costa *et al.* 2004). The first, pivotal phase leads to the establishment of the proximal–distal axis, which is reflected by the polarized localization of transcripts (Doan *et al.* 1996;

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Gomez *et al.* 2002; Drea *et al.* 2005; Ingouff *et al.* 2005a), and, in Arabidopsis, the cytological organization of syncytial endosperm domains (Boisnard-Lorig *et al.* 2001; Brown *et al.* 2003). These early events are most likely regulated by several maternally required genes (for example, Springer *et al.* 2000; Grini *et al.* 2002; Holding and Springer 2002), including members of the *Polycomb* Group complex (*Pc*-G). The identification and characterization of the Arabidopsis *Pc-G medea* (*mea*) mutant first demonstrated the principle of gametophytic maternal effects on seed development in plants (Grossniklaus *et al.* 1998). Mutations in *MEA* and in two other Arabidopsis *Pc*-G genes, *Fertilization Independent Endosperm* (*FIE*) (Ohad *et al.* 1996) and *Fertilization Independent Seed2* (*FIS*2) (Chaudhury *et al.* 1997), cause irregular nuclear proliferation in the unfertilized central cell as well as a delay in the developmental progression of the fertilized endosperm (Grossniklaus *et al.* 1998; Ingouff *et al.* 2005b). As a consequence of the latter, ectopic chalazal (posterior) endosperm-specific gene expression becomes manifest in inappropriately anterior positions (Sørensen *et al.* 2001; Guitton *et al.* 2004). Similarly, development of the chalazal endosperm is perturbed in two other Arabidopsis *Pc*-G mutants, *borgia* and *medicis/multicopy suppressor of IRA1* (Guitton *et al.* 2004). Parallel studies have demonstrated that alterations in the 2 maternal:1 paternal genomic balance in the endosperm also strongly influence early development of the Arabidopsis chalazal endosperm and the maize basal endosperm transfer layer (BETL). In both species, development of these domains is more dramatically perturbed in 2 maternal (m):2 paternal (p) endosperms derived from crosses between diploid females and tetraploid males (reviewed in Costa *et al.* 2004).

Here we report the comprehensive characterization of *baseless1* (*bsl1*)—a novel gametophytic maternal-effect mutant in maize. Before fertilization, *bsl1* mutants displayed defects in the central cell within the mature female gametophyte. The two polar nuclei were frequently displaced in *bsl1* central cells, yet, despite this irregularity fertilization was achieved, suggesting that sperm were able to efficiently locate and fuse with the displaced polar nuclei. Resulting mutant seeds displayed irregular distributions of transcripts specific to the basal region of syncytial and cellular endosperms. Mutant cellular endosperms also exhibited aberrant BETL development. BETL abnormalities were dramatically enhanced in seeds resulting from crosses between *bsl1*/+ females and wild-type tetraploid males. Our analysis of *bsl1* mutants provides new genetic evidence in plants that essential endosperm patterning component(s) are present in the central cell before fertilization.

MATERIALS AND METHODS

Plant material and growth conditions: The *baseless1* mutant was originally isolated from a self-pollination of a W22 inbred maize (*Zea mays*) plant carrying a novel mutable allele, *r1-m-Bolivia*, of the *R1* locus and was given the provisional designation *dex-4299** (Kermicle 1978). The mutant was typically propagated as heterozygous by transmission through the female and selection for viable *bsl1* defective kernels. Plants were grown in summer field conditions or in greenhouses under 16 hr light:8 hr dark cycles. *bsl1* heterozygous (*bsl1*/+) and wild-type (+/+) plants used for controls were grown under the same conditions for each experiment. For characterization of the effects of *bsl1* on seed development, *bsl1*/+ plants were pollinated with wild-type pollen. Segregating wild-type and mutant siblings were then taken from the same middle-third portion of the ear, as standard. With the exception of the mapping, all characterizations were performed in a standard W22 inbred background. Male transmission (Mt) of *bsl1* was calculated on the basis of both linkage to and transmission of a linked genetic marker. $Mt_{\text{linked marker}}$ equals the transmission of nonrecombinant *bsl1* pollen grains and recombinant *Bsl1*+ pollen grains. This was calculated as Mt_{bsl1} times the frequency of nonrecombinants $(1 - R)$ between *bsl1* and the marker plus the frequency of transmission of $Bsl1^{+}$ $(1 - Mt_{bsl1})$ times the frequency of recombinants between *bsl1* and the marker (R). Solving for Mt_{bsl1} gives $Mt_{bsl1} = (Mt_{\text{linked marker}} - R)/(1.0 - 2R)$, where R equals the recombination frequency between *bsl1* and the linked marker, and $Mt_{\text{linked marker}}$ equals the transmission of the allele linked to *bsl1*.

Mapping of *bsl1* was performed in crosses between *bsl1*/+, W22/W23 hybrid females and W23 wild-type males. Only *bsl1* defective kernels were used for the mapping population, as incomplete penetrance causes some normal kernels to carry *bsl1*, necessitating progeny testing to ensure that they were wild type. DNA was extracted from seedlings from these defective kernels and maize simple sequence repeat (SSR) markers were analyzed as described previously (Evans and Kermicle 2001).

Pollen tube growth analysis: Pollen was analyzed from five *bsl1*/+ heterozygotes and five homozygous wild-type plants. Old anthers were removed from plants the day before pollen collection. The following day, one newly extruded anther was picked from each plant prior to dehiscence, and anthers were quickly dissected to liberate pollen onto germination plates. *In vitro* pollen tube growth measurements were performed as described previously (Evans and Kermicle 2001).

Histology: For examination of pollen morphology, pollen grains were collected in the same manner as for pollen tube growth measurements, except that anthers were dissected directly on slides. Pollen was mounted under coverslips in iodide potassium iodine to visualize starch or stained with hematoxylin/ferric ammonium sulfate to visualize nuclei (Kindiger 1994). Pollen grains were analyzed with a Nikon Eclipse E600 microscope.

For analysis of embryo sacs, samples collected from homozygous wild-type and heterozygous *bsl1*/+ plants were fixed in 5% formaldehyde, 5% acetic acid, 45% ethanol under vacuum for 15 min, followed by overnight fixation in fresh fixing buffer at 4°. Samples were rinsed and stored in 70% ethanol. Ovules and developing kernels were bisected along the longitudinal axis of the ear, dehydrated through a standard ethanol series, and cleared in methyl salicylate. Samples were mounted in methyl salicylate and visualized on a Bio-Rad (Hercules, CA) laser scanning confocal microscope. Excitation was performed at 488 nm and emission was collected at both 522- and 585-nm wavelengths and combined. Image files were opened with NIH Image v. 1.62 and handled in Adobe Photoshop v. 5.0.

For epifluorescence microscopy of early endosperms, kernels were collected from wild-type ears and ears segregating for the *bsl1* mutation at 1–3 days after pollination (dap) and then fixed as described by Costa *et al.* (2003). DNA content of nuclei from 3-dap wild-type and *bsl1* endosperms was measured using a Newcastle Photometric Systems photon

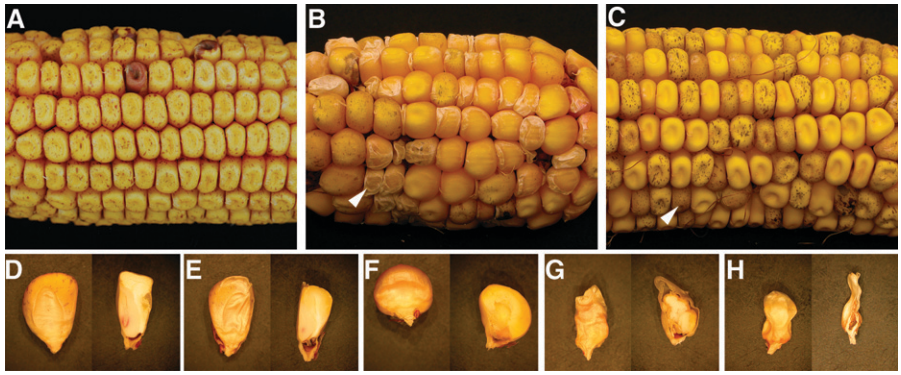

FIGURE 1.—The *bsl1* seed phenotype. (A) Ear from a homozygous wild-type plant pollinated with pollen from a *bsl1*/+ heterozygous plant, exhibiting normal kernels. (B) Ear from a *bsl1*/+ heterozygous female pollinated with homozygous wild-type pollen, showing a strong *bsl1* maternal effect. Approximately half of the kernels have reduced or aborted endosperms with loose pericarps (arrowhead). (C) Ear from a *bsl1*/+ heterozygous female pollinated with homozygous wild-type pollen, showing a mild *bsl1* maternal effect. Approximately half of the kernels have slightly reduced endosperms (arrowhead). (D–H) Phenotypic classes of kernels produced in test crosses and self-crosses of *bsl1*/+ plants (germinal face of kernels on the left and median longitudinal sections of kernels on the right). The germinal face of sectioned kernels is oriented to the left for all kernels. (D) Wild type. (E–H) *bsl1* phenotypic classes. (E) Reduced endosperm. (F) Germless. (G) Loose pericarp. (H) Empty pericarp.

counting photometer attached to a side port of a Nikon Diaphot TMD inverted microscope. Samples were viewed with a Zeiss 100× oil immersion objective using an ultraviolet filter set with a excitation filter (360 nm, bandpass 10 nm), a dichroic mirror (400 nm), and a barrier filter (420 nm). For each measurement, individual calibration curves were prepared on the basis of a linear regression analysis as the relative fluorescence units (RFU) of DAPI-stained nuclei in proportion to DNA content (COLEMAN and GOFF 1985).

mRNA *in situ* hybridization: *In situ* hybridization was performed on kernels as described previously (COSTA *et al.* 2003). Probes for MRP1 (GOMEZ *et al.* 2002) and the maize homolog of barley END1 (DOAN *et al.* 1996) were generated by reverse transcriptase amplification (RT–PCR) with gene-specific oligonucleotides: MRP1.FOR 5′-ATGAATCCCAACTTCAAC AGTGTGTG-3′, MRP1.REV 5′-TATCGGTTA TATATCTGGCT CTCC-3′, END1.FOR 5′-ATGAAACGAGAGTGCAAGCAGTT TGAG-3′, and END1.REV 5′-CATACTAAGAGGAACTGTAT AACTCC-3′. Slides were viewed with a Zeiss Axiophot microscope under bright field optics and images were digitally recorded with a coolpix Nikon camera.

Transgenic reporter gene analysis: To analyze BETL-specific reporter gene expression in mutant and wild-type seeds, *bsl1*/+ plants were crossed as females by males carrying one of two BETL transgenic reporters: Pro_{Bet1}*:GUS* (HUEROS *et al.* 1999a) or Pro_{Meg1}*:GUS* (GUTIERREZ-MARCOS *et al.* 2004). Kernels were cut along the longitudinal axis and stained for GUS activity as previously described (COSTA *et al.* 2003; GUTIERREZ-MARCOS *et al.* 2004). To analyze the combined effects of *bsl1* and parental genomic imbalance in the tetraploid endosperm on BETL-specific reporter gene expression, plants heterozygous for *bsl1* and homozygous for Pro_{Meg1}*:GUS* transgene were crossed by wild-type (W22) tetraploid plants.

RESULTS

***baseless1* is a novel maternal-effect mutation in maize:** The *baseless1* mutant arose spontaneously as a sector on a female inflorescence (ear) of a wild-type W22 inbred maize plant. One side of the ear had 70 normal seeds; the other side of the ear had 40 defective seeds and 58 normal seeds. Fortuitously, the viability of some abnormal seeds allowed recovery and propagation of the mutant when transmitted maternally. *bsl1* was initially mapped to chromosome 5 using *waxy1* (*wx1*)-marked reciprocal translocations. This location was confirmed by linkage to the *purple aleurone1* (*pr1*) gene (49/226 = 21.7%; $P < 0.0001$). Mapping with SSR markers placed *bsl1* on the short arm of chromosome 5 in proximity to the centromere—between *umc1110* and *bnlg603*.

After pollinating *bsl1*/+ plants with wild-type pollen, 45.7 ± 2.6% of the seeds were visibly defective (Figure 1 and Table 1). Progeny testing of phenotypically normal seeds revealed that 8.4 ± 2.3% were also carrying the *bsl1* defective allele, so that the percentage of *bsl1*/+ kernels was always ~50% (Table 1). Progeny testing of the defective seeds revealed that they almost exclusively (238/241 individuals) segregated defective kernels in the next generation (*i.e.*, due to *bsl1* inheritance). Further, the penetrance and severity of the *bsl1*/+ seed phenotype varied from cross to cross within the same genetic background (Figure 1, B and C), suggesting that the mutant phenotype is most likely influenced by extrinsic environmental factors. Mature mutant seeds fell into different phenotypic classes, including reduced endosperm, loose pericarp, empty pericarp, and germless (Figure 1, D–H). Consequently, the most severely affected seeds failed to germinate (27/98), while those that did displayed root or shoot defects (18/71), including fasciations and/or absence of the root and shoot axes.

When wild-type females were crossed with *bsl1*/+ heterozygous males, the frequency of defective kernels did not significantly differ from that observed in wild-type crosses (Table 1). These findings indicate that *bsl1* male transmission alone has no detrimental effect on seed development and that *bsl1* is not dominant. Moreover, progeny testing of the offspring of *bsl1*/+ males revealed that the *bsl1* mutant allele was transmitted through pollen less efficiently than the wild-type allele. The rate of *bsl1* male transmission was calculated as 12.0 ± 2.3%, both by progeny testing *bsl1*/+ males and by segregation distortion of the linked marker *pr1* or a

TABLE 1

Phenotypic classes of kernel progeny from pollinations with wild-type and *bsl1*/+ plants

Type of cross	Defective +/+ and *bsl1*/+ (%)	Morphologically normal *bsl1*/+ (%)	Morphologically normal +/+ (%)
wt × wt (844 kernels/4 ears)	2.4 ± 1.1	0	97.6 ± 1.1
bsl1/+ × wt (1282 kernels/8 ears)	45.7 ± 2.6	8.4 ± 2.3[a,b]	45.9 ± 0.8
wt × *bsl1*/+ (1719 kernels/11 ears)	2.0 ± 0.7	12.0 ± 2.3[a,b]	86.0 ± 2.4
bsl1/+ × *bsl1*/+ (1414 kernels/8 ears)	45.3 ± 1.4	14.4 ± 1.2[a]	40.3 ± 2.0

Percentages given are the average of each kernel class per cross ± the standard error of the mean.

[a] In some crosses the transmission of *bsl1* was calculated from the transmission and linkage of the genetic markers *purple aleurone1* (*pr1*), or a *waxy1* (*wx1*) marked reciprocal translocation T5-9c, linked in repulsion phase to *bsl1*. Male transmission rates of *bsl1* were calculated as $Mt_{bsl1} = (Mt_{\text{linked marker}} - R)/(1-2R)$ (see MATERIALS AND METHODS).

[b] In those crosses without an endosperm marker, the transmission rate of *bsl1* in morphologically normal kernels was inferred by sample progeny testing.

waxy1 (*wx1*) marked reciprocal translocation, T5-9c *wx1* (Table 1). These data indicate that the deficit of *bsl1* heterozygotes after male transmission was caused by reduced transmission of the *bsl1* allele rather than by frequent reversion of *bsl1* to wild type.

We self-pollinated *bsl1*/+ heterozygotes in an attempt to generate *bsl1/bsl1* homozygous progeny. If *bsl1* homozygous seeds were viable, they would be expected to occur at an average frequency of 6% (*i.e.*, on the basis of the probability of 12% male transmission and 50% female transmission of *bsl1* alleles). Furthermore, 12% of the resulting defective kernels (*i.e.*, those with maternal *bsl1*) are predicted to have inherited paternal *bsl1* and be homozygous. With this in mind, all resulting defective kernels were selected, and plants from these seeds were grown to maturity. These plants did not exhibit any morphological abnormalities, and they were subsequently progeny tested for homozygosity of *bsl1* on the basis of the frequency of defective seed production (*i.e.*, from ~50% defective seeds to ~100% defective seeds). However, of 103 plants tested, none were homozygous for *bsl1*, indicating that the homozygotes were seed lethal (likelihood that *bsl1/bsl1* plants were missed by random chance, $P < 0.0001$). No novel kernel phenotypes were visible in self-pollinations of *bsl1*/+ heterozygotes compared to pollinations of *bsl1*/+ females by wild type, suggesting that the *bsl1/bsl1* individuals are not phenotypically distinct from heterozygous individuals carrying maternal *bsl1* alleles. To confirm this finding we carried out test crosses and self-pollinations of *bsl1 Wx1*$^+$*/Bsl1*$^+$ T5-9c *wx1* plants, which enabled us to distinguish kernels carrying wild-type or *bsl1* alleles. Progeny derived from self-pollinations of *bsl1*/+ plants exhibited a higher proportion of lethal phenotypic classes (18.9%) compared to those derived from test crosses (7%) (Table 2). Therefore the rise in seed lethality following self-pollinations can be accounted for by the presence of *bsl1* homozygotes in these populations.

Taken together, these genetic data indicate that *bsl1* confers a maternal effect on seed development. That only half of the seeds are affected is consistent with

TABLE 2

Phenotypic classes of kernels produced in test crosses and self-crosses of *bsl1*/+ plants marked with T5-9c *wx1* in repulsion to *bsl1*

		bsl1/+ and *bsl1/bsl1*[b]					+/+:
Ear parent	Pollen parent	Empty pericarp[a] (%)	Loose pericarp[a] (%)	Germless[a] (%)	Reduced endosperm (%)	Normal morphology (%)	Normal morphology (%)
bsl1 Wx1$^+$ + T5-9c *wx1* (199 kernels/3 ears)	*wx1*	1.9	3.5	1.6	33.6	12.8	46.6
bsl1 Wx1$^+$ + T5-9c *wx1* (352 kernels/5 ears)	*bsl1 Wx1*$^+$ + T5-9c *wx1*	3.2	10.3	5.4	28.1	8.7	43.9

bsl1 phenotypic classes are listed from most to least severe (empty pericarp > loose pericarp > germless > reduced endosperm > normal).

[a] Lethal phenotypic classes.

[b] Include +/+ defective kernels.

TABLE 3

Phenotypes of pollen from wild-type and *bsl1*/+ plants

	Pollen morphology		*In vitro* pollen analysis	
Plant genotype	Aborted (%)	Normal (%)	Germination frequency (after 30 min) (%)	Pollen tube length (after 3.5 hr) (μm)
Wild type	8.5 ($n = 398$)	91.5 ($n = 398$)	65 ($n = 834$)	463 ± 13[a] ($n = 168$)
bsl1/+	8.0 ($n = 387$)	92.0 ($n = 387$)	67 ($n = 397$)	451 ± 12[a] ($n = 212$)

[a] ±1 standard error of the mean.

either a gametophytic maternal effect or an incompletely penetrant sporophytic maternal effect. The correlation between kernel phenotype and the inheritance of *bsl1* in the progeny of these crosses demonstrates that the presence of the mutation in the embryo sac rather than the diploid parent is critical, thus supporting the view that the *bsl1* maternal effect is gametophytic.

***baseless1* affects development of the central cell within the female gametophyte:** The reduced male transmission of *bsl1* and linked markers from *bsl1*/+ heterozygotes suggests that mutant *bsl1* pollen grains are at a competitive disadvantage to wild-type pollen. Thus it is possible that *bsl1* causes either an increased frequency of aborted and abnormal pollen or more subtle defects, such as in pollen tube growth rate, its ability to target embryo sacs, or the fertilization process, any of which could result in *bsl1* pollen grains achieving less efficient fertilization than wild type. We therefore examined pollen from heterozygous *bsl1*/+ plants and compared them to pollen from wild-type plants (see MATERIALS AND METHODS). No abnormalities in pollen morphology, starch filling, or nuclear number were apparent in pollen from *bsl1*/+ heterozygous plants (Table 3), suggesting that *bsl1* pollen grains develop normally to maturity. Further, germination and tube growth rates of pollen from *bsl1*/+ heterozygous plants and wild-type plants were compared *in vitro* (see MATERIALS AND METHODS) and were found to be identical (Table 3), indicating that pollen tube growth is not perturbed by *bsl1*.

We also investigated the possible effects of the *bsl1* mutation on female gametophyte development. Cell number, position, and morphology within embryo sacs of *bsl1*/+ plants were as in wild type, with discrepancies observed only in the central cell. In wild-type plants, the two polar nuclei of the central cell were always positioned adjacent to the egg cell, along the central longitudinal axis of the embryo sac (Figure 2A). However, in *bsl1*/+ plants we found that the two polar nuclei were not properly positioned within the central cell in 38% ($n = 142$) of embryo sacs examined (Table 4). In 16.9% of embryo sacs, the two polar nuclei were found apposed to the abgerminal wall of the central cell, whereas the polar nuclei were situated proximal to the adgerminal wall in only 0.7% of embryo sacs (Figure 2B, Table 4). In contrast, the two polar nuclei were situated off center, in that they were abgerminal to the central longitudinal axis of the central cell, in 20.4% of embryo sacs from *bsl1*/+ heterozygotes—a phenotype rarely seen in wild-type plants (Figure 2C). In all cases, the central cytoplasmic strands that extend from the antipodal cells to the polar nuclei, and from the polar nuclei to the egg, were also displaced in mutant embryo sacs compared to wild type. Because the observed frequency of abnormal embryo sacs in *bsl1*/+ heterozygotes was significantly lower than the expected 50% ($\chi^2 = 8.14$,

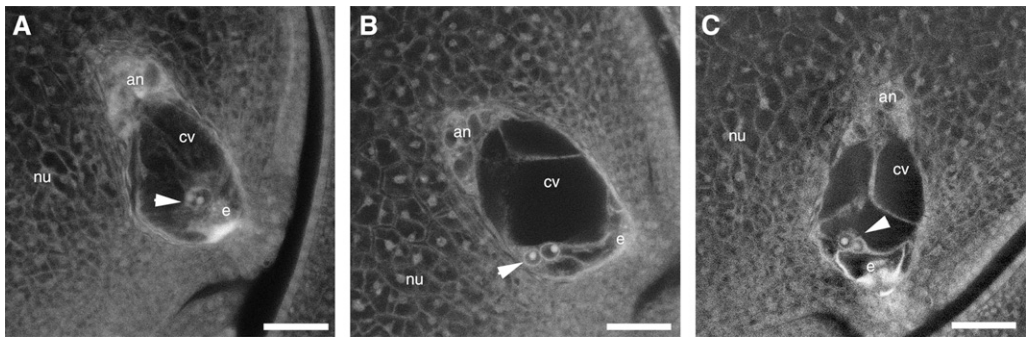

FIGURE 2.—Embryo sac morphologies in *bsl1*/+ plants. (A) Wild-type embryo sac with the two central cell polar nuclei centrally located above the egg cell. (B) *bsl1* mutant embryo sac with the polar nuclei located adjacent to the abgerminal wall of the central cell. (C) *bsl1* mutant embryo sac showing polar nuclei situated off center, abgerminal to the central longitudinal axis. Arrowheads point to the polar nuclei. an, antipodals; cv, central vacuole; e, egg cell; nu, nucellus. Bar, 25 μm.

TABLE 4

Position of central cell polar nuclei in embryo sacs of wild-type and *bsl1*/+ plants

Plant genotype	Central	Apposed to abgerminal wall	Off center, abgerminally positioned	Apposed to adgerminal wall
Wild type	85	0	6	0
bsl1/+	88	24	29	1

$P < 0.01$), we predicted that 12% of embryo sacs must have been defective for *bsl1*, yet were indistinguishable from wild type (*i.e.*, 24% of *bsl1* embryo sacs are normal in appearance). Therefore the effects of *bsl1* on polar nuclei positioning are not fully penetrant. Interestingly, we also observed that 31.8% ($n = 91$) of embryo sacs from +/+ W22 plants contained polar nuclei that had fused prior to fertilization and that 31.6% ($n = 142$) of embryo sacs from *bsl1*/+ W22 plants also contained fused polar nuclei—a phenomenon that has not been previously reported in maize.

Given that a significant number of maternal-effect mutations cause precocious endosperm development in the absence of fertilization, we investigated whether *bsl1* had a similar effect. Following examination of mature, nonfertilized embryo sacs from 20 *bsl1*/+ plants, we found no evidence of abnormal nuclear proliferation or autonomous endosperm development. On the other hand, controlled pollinations of embryo sacs from heterozygous *bsl1*/+ plants revealed that they could be fertilized as efficiently as in wild type, despite the displacement of polar nuclei in some mutant embryo sacs. Thus, *bsl1* is required for correct development of the central cell within the female gametophyte. Despite the low male transmission of *bsl1*, the mutation does not appear to affect male gametophyte morphology, suggesting that *bsl1* pollen grains might instead be defective in a later critical function, such as interaction with the stigma (silk), targeting to the embryo sac, or delivery of the sperm cells.

Mutation in *bsl1* causes maternal-effect abnormalities on seed development: Because *bsl1* causes a maternal effect on seed development, we determined the stage at which developmental abnormalities were first manifest in mutant kernels. For this, we analyzed 1- and 2-dap embryo and syncytial endosperm sections from wild-type plants and plants carrying the *bsl1* mutation. However, no abnormalities were detected in embryos or in number of nuclei and their arrangements within syncytial endosperms at these stages (data not shown). In wild-type maize, syncytial endosperms commonly undergo coordinated cellularization, initiating from the periphery of the syncytium and progressing centripetally until a fully cellular structure is formed (Costa *et al.* 2003). In this study, fully cellular wild-type endosperms were formed by 3 dap (Figure 3A). By contrast, ~40% of 3-dap endosperms examined from *bsl1*/+ plants exhibited delayed cellularization, which was indicated by the continued presence of the central vacuole at a time when other sibling endosperms were fully cellular

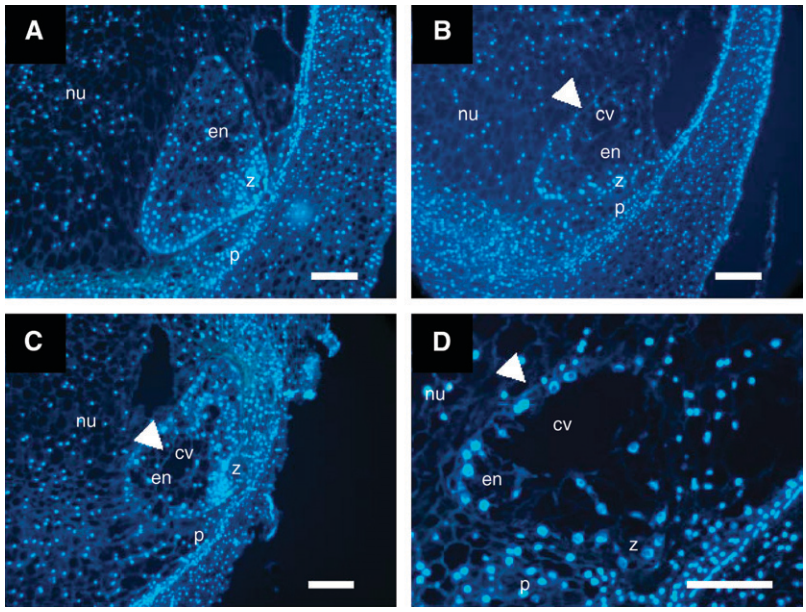

Figure 3.—Developing 3-dap endosperms segregating from *bsl1*/+ plants pollinated with wild-type pollen. (A) Wild-type fully cellular endosperm. (B–D) Segregating *bsl1* mutant endosperms showing delayed cellularization—as indicated by the prolonged presence of the central vacuole (arrowheads), occurring irregularly, in a noncentripetal fashion. Endosperm sections were stained with DAPI and viewed under fluorescence microscopy. cv, central vacuole; en, endosperm; nu, nucellus; p, pericarp; z, zygote. Bar, 50 μm.

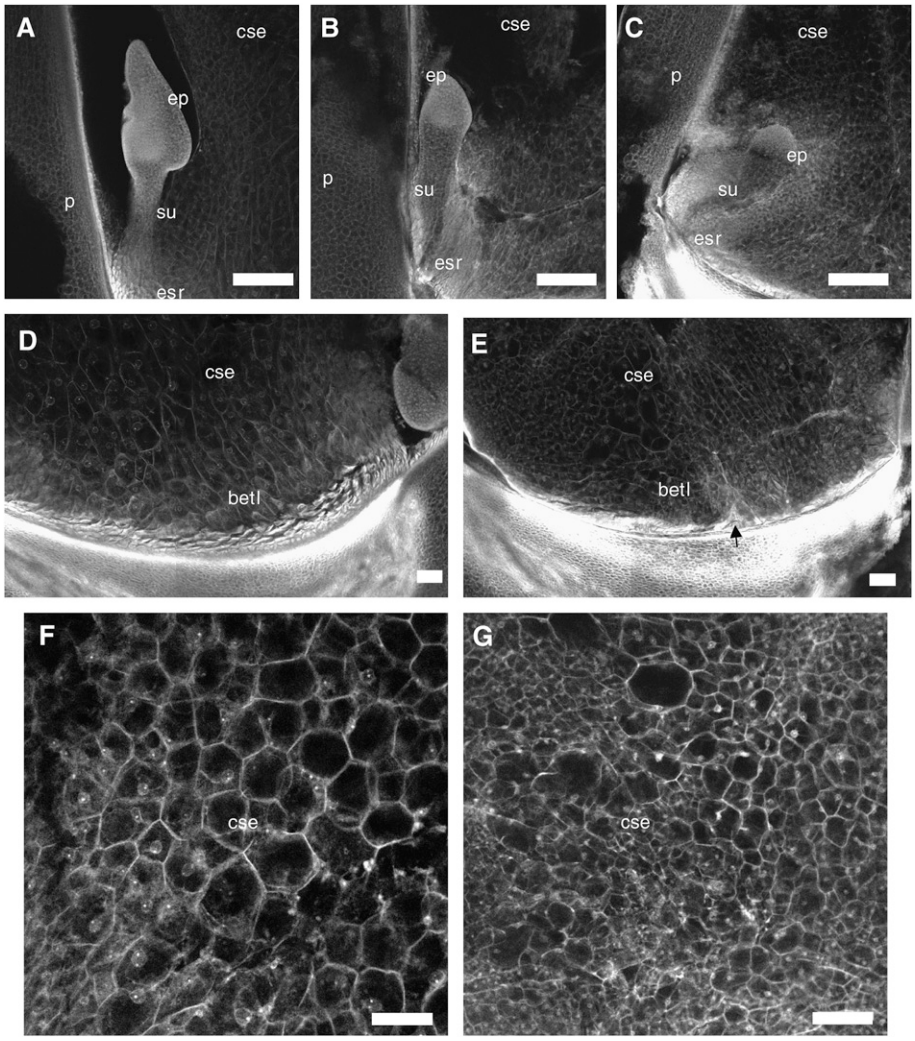

FIGURE 4.—Confocal microscopy analysis of 9-dap embryos and endosperms from *bsl1*/+ plants pollinated with wild-type pollen. (A, D, and F) Wild type. (B, C, E, and G) *bsl1*. (A) Wild-type coleoptile stage embryo with prominent shoot apical meristem and (B and C) sibling mutant embryos lagging at the transition stage. (D and E) BETL endosperm morphology. (D) Wild-type endosperm with BETL consisting of approximately three layers of slightly elongated cells (autofluorescent) located above the pedicel (intense autofluorescent signal). (E) Typical *bsl1* endosperm possessing an irregular BETL containing fewer cell layers. Arrow points to region of basal endosperm devoid of BETL. (F and G) Starchy endosperm morphology. Cells in the central endosperm are large and regularly spaced in wild-type endosperms (F), but appear smaller and of variable size in *bsl1* endosperms (G). betl, basal endosperm transfer layer; cse, central starchy endosperm; ep, embryo proper; esr, embryo surrounding region; p, pericarp; su, suspensor. Bar, 100 μm.

(Figure 3, B–D). To determine the ploidy level of these endosperms, we performed a microspectrofluorometric analysis. We found no differences in the nuclear fluorescence intensity (RFU) of these endosperms, indicating that the ploidy levels of sibling wild-type (145.5 ± 5.3 RFU, $n = 98$) and *bsl1* mutant (152.6 ± 4.5 RFU, $n = 127$) endosperms were similar.

By 9 dap, *bsl1* mutant kernels were macroscopically distinguishable from their wild-type siblings. We therefore examined the cellular organization of 9-dap sibling mutant and wild-type kernels from *bsl1*/+ plants to determine which tissues were most affected by the mutation at this stage. Our 9-dap wild-type embryos were at the coleoptile developmental stage (Figure 4A), whereas mutant sibling embryos were retarded and had reached only the transition stage (Figure 4, B and 4C). These embryos typically possessed a morphologically normal suspensor and embryo proper (Figure 4B), although some of the more severe mutants had a smaller than typical embryo proper (Figure 4C).

In mutant endosperms, the most notable and consistent defects were observed in the organization of the BETL. Wild-type endosperms regularly possessed several layers of elongated and angular BETL cells in the basal region (Figure 4D), whereas we observed patchy distribution of BETL-like cells across fewer cell layers along the basal endosperm of mutant kernels (Figure 4E). Interestingly, *bsl1* mutants displayed subtle or no morphological defects in the epidermal aleurone layer or embryo surrounding region (ESR) (data not shown). Abnormalities were, however, noted in the central starchy endosperm (CSE) in more severely perturbed *bsl1*/+ kernels, which contained smaller and more irregularly sized cells compared to wild type (Figure 4, F and G).

In summary, our findings show that after fertilization, *bsl1* causes retarded growth of embryo and endosperm. Furthermore, *bsl1* predominantly confers aberrations to the BETL tissue in mutant endosperms.

***baseless1* alters the spatial distribution of transcripts specific to the basal syncytial and cellular endosperm:** To further investigate the effects of the *bsl1* mutation on early endosperm development, we performed mRNA *in situ* hybridization analysis of known transcripts

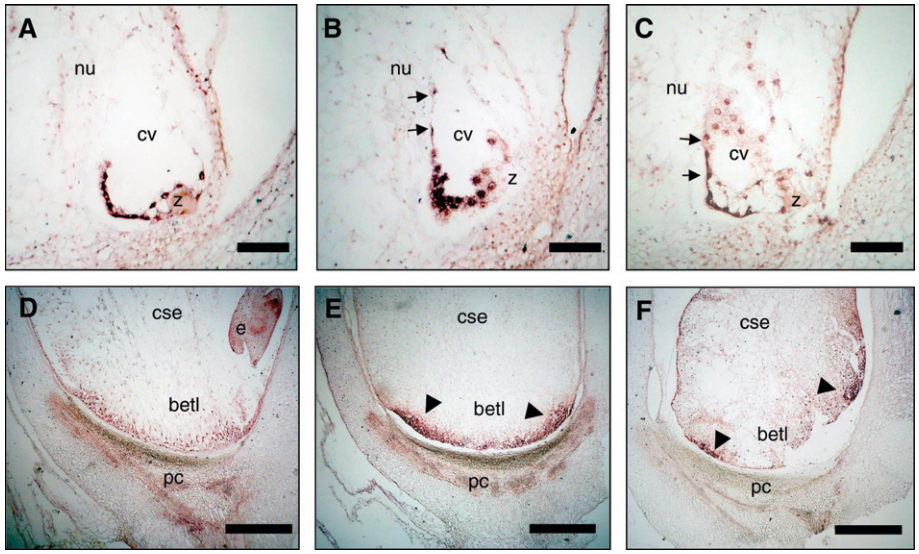

FIGURE 5.—mRNA *in situ* hybridization of basal endosperm-specific transcripts in sibling wild-type and mutant kernels. (A–C) 2-dap syncytial endosperms. (D–F) 9-dap cellular endosperms. (A) *ZmEND1* transcript is confined to the basal portion of the wild-type free-nuclear endosperm. (B and C) In sibling mutant endosperms, *ZmEND1* expression is displaced from the basal portion of the syncytial endosperm to more lateral regions (arrows). (D) Wild-type *meg1* expression is restricted to the BETL, whereas in *bsl1* endosperms (E and F) *meg1* transcript is localized in discrete areas of the endosperm (arrowheads). betl, basal endosperm transfer layer; cse, central starchy endosperm; e, embryo; pc, placentochalazal region; cv, central vacuole; nu, nucellus; z, zygote. Bar, 100 μm.

expressed in the syncytial endosperm. We examined the localization of two basal-specific transcripts, maize *end1* (see MATERIALS AND METHODS) and *MRP1* (GOMEZ *et al.* 2002), in wild-type syncytia and in syncytia isolated from ears segregating the *bsl1* mutant allele. Maize *end1* transcript was strongly detected along the entire basal portion of syncytial endosperms from wild-type plants (Figure 5A). By contrast, *end1* transcript was localized in the basal region of ~50% ($n = 78$) of syncytial endosperms examined from plants segregating for *bsl1*. The remainder exhibited distribution of *end1* transcript either in patches along the basal portion of the syncytium or in the basal medio-lateral margins of the syncytial endosperm (Figure 5, B and C). We found similar results with MRP1, which was weakly expressed in both wild-type and mutant syncytial endosperms examined (data not shown).

In situ hybridization was also performed on 9-dap cellular endosperms to detect a range of transcripts specific to the different domains. Our analysis revealed that several transcripts specific to the aleurone and ESR were correctly localized in wild-type and mutant sibling kernels (data not shown). In stark contrast, we discovered that BETL-specific transcripts were not properly localized in mutant endosperms. In wild type, *meg1* transcript was detected throughout the BETL tissue (Figure 5D), whereas in *bsl1* mutants, this transcript was present only in portions of the abgerminal and adgerminal basal endosperm (Figure 5, E and F). Thus our data indicate that basal endosperm-specific gene expression is severely perturbed during free-nuclear and cellular endosperm development by mutation in *bsl1*.

***Baseless1* is necessary for the establishment of correct BETL-patterning components:** Previous work in maize has shown that interploidy crosses interfere with the normal postfertilization developmental program of the BETL (CHARLTON *et al.* 1995; GUTIERREZ-MARCOS *et al.* 2003). We therefore decided to cross diploid wild-type and *bsl1*/+ plants as female by wild-type tetraploid plants to investigate whether the maternal defects caused by *bsl1* could lead to further alterations in BETL development in resulting tetraploid endosperms. By following this strategy, we predicted one of three different outcomes: the expected defects in the BETL of tetraploid *bsl1/bsl1/+/+* endosperms would be similar to those observed in triploid *bsl1/bsl1/+* endosperms, be enhanced, or be suppressed. To facilitate this analysis, *bsl1/+* plants were initially crossed as females by plants carrying one of two BETL-specific promoter GUS fusions, Pro_{Meg1}*:GUS* (GUTIERREZ-MARCOS *et al.* 2004) or Pro_{Bet1}*:GUS* (HUEROS *et al.* 1999a). In wild-type kernels resulting from crosses between *bsl1/+* female heterozygotes and Pro_{Meg1}*:GUS*-carrying plants, uniform GUS staining was observed in several layers of the BETL (Figure 6A). As expected from our *in situ* analysis of basal-specific endosperm transcripts, mutant sibling kernels displayed small patches of GUS precipitate in the lateral margins of the basal endosperm, denoted "mirror image" (Figure 6B). Similar GUS staining patterns were observed when using pollen from transgenic lines carrying the Pro_{Bet1}*:GUS* reporter to pollinate *bsl1/+* plants (data not shown). *bsl1/+*, Pro_{Meg1}*:GUS* and *+/+*, Pro_{Meg1}*:GUS* diploid females were then crossed by tetraploid males and resulting tetraploid endosperms were stained for GUS at different developmental stages. From ears carrying only wild-type *Bsl1* alleles, all kernels were morphologically similar and GUS staining was often present in discrete clusters along the basal, and in some instances apical, endosperm and denoted "top–bottom" (Figure 6C). By contrast, three distinct classes of seed phenotype were found in ears segregating the *bsl1* defective allele. Kernels of the first class (top–bottom: 55.8 ± 6.4%, $n = 691$) were similar to kernels examined from plants carrying *Bsl1* wild-type alleles (Figure 6D). In contrast, kernels of the second and third classes (44.1 ± 3.1%) were reduced in size and showed

much more unusual and varied GUS staining patterns. We therefore assumed that these kernels were defective for *bsl1*. The majority of these kernels (25.5 ± 4.1%) exhibited small patches of GUS staining in the basal endosperm (mirror image) and, at times, in the apical endosperm (Figure 6E), while others (18.6 ± 6.5%) displayed intensely staining puncta scattered throughout the entire endosperm (Figure 6F). Additionally, we noted that the more dramatic GUS staining patterns were observed in the most severely reduced endosperms. Taken together, these findings suggest that not only do the prefertilization defects caused by *bsl1* greatly perturb BETL development, but also these abnormalities are enhanced in the tetraploid endosperm.

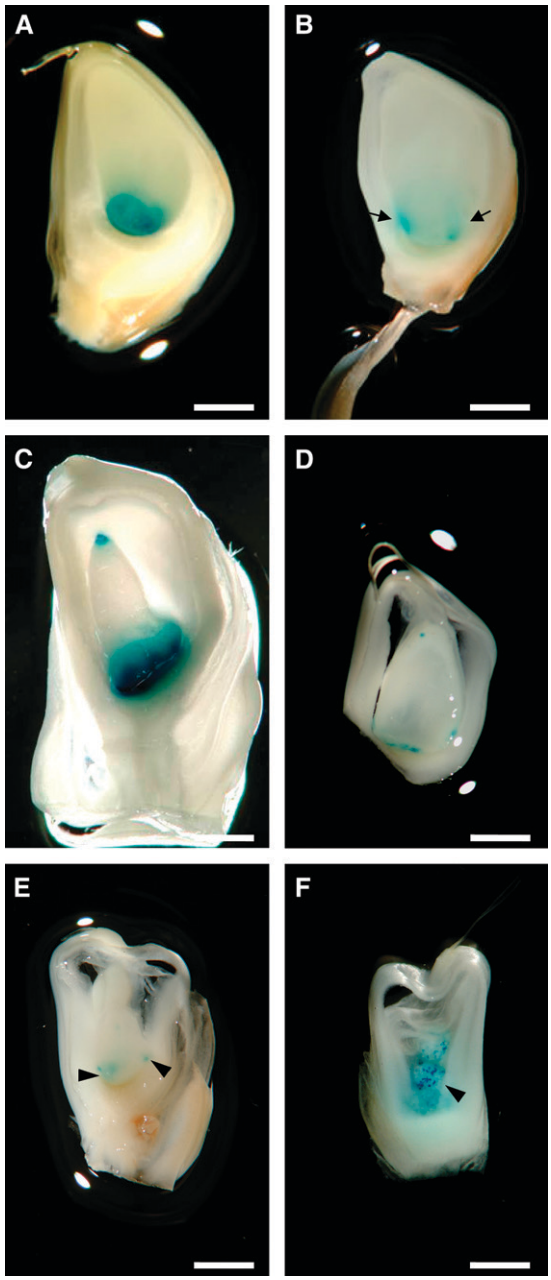

Figure 6.—Effects of *bsl1* on BETL-specific transgenic reporter expression in triploid and tetraploid endosperms. (A and B) 10-dap sibling kernels taken from a *bsl1*/+, Pro_{Meg1}*:GUS*/Pro_{Meg1}*:GUS* plant pollinated by a wild-type diploid plant. (A) Wild-type triploid endosperm with uniform Pro_{Meg1}*:GUS* expression (blue) distributed along the basal endosperm. (B) Typical mirror-image GUS expression confined to discrete basal areas of a typical *bsl1*/*bsl1*/+ endosperm (indicated by arrows). (C) 10-dap tetraploid endosperm expressing Pro_{Meg1}*:GUS* in the basal and apical endosperm (denoted "top–bottom"). Tetraploid endosperms were generated from crosses between wild-type Pro_{Meg1}*:GUS*/Pro_{Meg1}*:GUS* diploid plants and wild-type tetraploid plants. (D–F) Three distinct classes of 10-dap sibling kernels segregating in an ear from a *bsl1*/+, Pro_{Meg1}*:GUS*/Pro_{Meg1}*:GUS* plant pollinated by a wild-type tetraploid plant. (D) Typical kernel of the first class showing top–bottom Pro_{Meg1}*:GUS* expression patterns similar to those found in tetraploid endosperms shown in C. (E) Abnormal kernel of the second class exhibiting irregular mirror-image GUS staining patterns in the basal endosperm. (F) Kernel typical of the third class showing highly scattered Pro_{Meg1}*:GUS* staining throughout the endosperm (arrowheads). Bar, 200 μm.

DISCUSSION

***bsl1* is a novel gametophytic mutant in maize:** To gain further understanding of plant reproductive development, efforts have been made in recent years to identify mutations affecting development and function of the male and female gametophytes (Feldmann *et al.* 1997; Christensen *et al.* 1998, 2002; Howden *et al.* 1998; Ebel *et al.* 2004; Johnson *et al.* 2004; Pagnussat *et al.* 2005). For instance, a range of mutations affecting aspects of pollen development, such as the first asymmetric mitotic division, pollen tube growth, or sperm delivery, have been identified thus far (reviewed in McCormick 2004).

Our analysis of *bsl1* mutants revealed that transmission of the *bsl1* mutant allele through the male was reduced to 12%, which would imply that male gametophyte development and/or function is perturbed by mutation in *bsl1*. However, no obvious cellular defects in pollen grain morphology or any deficiency in pollen tube growth were detected, suggesting that *bsl1* pollen is not subject to a fundamental developmental or metabolic defect. Instead, we postulate that *bsl1* male gametophyte function might be affected either during pollination (*e.g.*, interaction of mutant pollen with silks) or during fertilization (*e.g.*, in delivery and/or function of the sperm cells), where, similarly to maize *rop2*, *maternal effect lethal1*, and *aberrant pollen transmission1* mutant pollen (Evans and Kermicle 2001; Arthur *et al.* 2003; Xu and Dooner 2006), it is at a competitive disadvantage. By contrast, we noted that *bsl1* caused abnormal development of the female gametophyte. Thus far, an increasing number of female gametophytic mutations affecting most stages of embryo sac development, such as general nuclear division and migration, cellularization, and/or fusion of the polar nuclei, have been identified (Moore *et al.* 1997; Christensen *et al.* 1998, 2002;

Ebel *et al.* 2004; Pagnussat *et al.* 2005). The majority of these mutations consequently impair fertilization. Because correct development of the egg apparatus (especially the synergids) is key to successful fertilization (Higashiyama *et al.* 2001; Huck *et al.* 2003; Rotman *et al.* 2003; Marton *et al.* 2005), it is likely that the egg apparatus is unaffected by the *bsl1* mutation, as fertilization of *bsl1* embryo sacs is achieved, although subtle defects cannot be ruled out. However, we found notable defects in *bsl1* central cells, which exhibited abnormalities in the position of the polar nuclei, while other cells of the female gametophyte did not show any such phenotypes. Interestingly, the proportion of fused polar nuclei in embryo sacs from wild-type and *bsl1*/+ plants was similar, as was the fertilization efficiency of wild-type and mutant central cells, together suggesting that most central cell functions are not impaired by the *bsl1* mutation. Given that the penetrance of the misplacement of polar nuclei in mutant central cells was only 38% ($n = 142$), it is probable that the *bsl1* maternal effect on seed development is not a direct consequence of misplacement of the polar nuclei *per se*. Instead, this result points to underlying developmental abnormalities in the *bsl1* central cell, which lead to both the maternal effect and misplacement of the polar nuclei. Nonetheless, it is unclear how the sperm are able to locate and fuse with the misplaced polar nuclei in *bsl1* embryo sacs as efficiently as in wild type. While a great deal of experimental evidence points to an active role of the female gametophyte in targeting the pollen tube to the synergids for sperm cell release, very little is known about how the sperm nuclei are subsequently targeted to the egg and central cell nuclei to achieve karyogamy after fertilization. One possibility is that this second step is a passive process that depends on the surrounding cytoskeleton to effectively deliver the sperm cells to the appropriate nuclear positions within the embryo sac, a process that in turn is dependent on embryo sac architecture. The displaced cytoplasmic strands in *bsl1* central cells support this view, although an alternative explanation of there being an active attraction between the sperm and central cell nuclei cannot be ruled out.

Maternal-effect *baseless1* predominantly confers developmental abnormalities to the basal endosperm transfer tissue: The *bsl1* mutation caused ~50% seed abnormalities when plants carrying the *bsl1* defective allele were crossed by wild-type pollen, but not when wild-type plants were crossed by *bsl1* pollen, indicating that the *bsl1* mutation has a maternal effect on seed development rather than a dominant effect. Maternal effects can be caused by mutations in genes expressed either in the surrounding sporophytic tissue (for examples see Felker *et al.* 1985; Ray *et al.* 1996; Colombo *et al.* 1997) or in the female gametophyte [examples include *capulet1* and *capulet2* mutants (Grini *et al.* 2002), *prolifera1* (Springer *et al.* 2000; Holding and Springer 2002), and *maternal effect lethal1* (Evans and Kermicle 2001)] and whose gene products are necessary for correct development of embryo and endosperm. The cosegregation of *bsl1* alleles with the defective kernels demonstrates maternal gametophytic—rather than sporophytic—inheritance.

A distinct class of maternal-effect genes comprises members of the *Pc*-G complex. *MEA*, *FIE*, and *FIS2* are expressed in the central cell before fertilization, where they are required for suppression of autonomous endosperm development until fertilization occurs (Ohad *et al.* 1996; Chaudhury *et al.* 1997; Grossniklaus *et al.* 1998). During early endosperm development these genes are maternally expressed and paternally silenced. Mutant *mea*, *fie*, and *fis2* endosperms all exhibit retarded growth, while embryos are inviable. Similar to *bsl1* mutant endosperms, the region thought to be responsible for nutrient transfer is also affected in *mea*, *fie*, and *fis2* endosperms (Sørensen *et al.* 2001; Guitton *et al.* 2004; Ingouff *et al.* 2005b). However, the nature of the aberrant maternal effects of *bsl1* on seed development differs from that reported for defects in *Pc*-G class mutants, in that *bsl1* does not induce parthenogenic development of the endosperm. In addition, when *bsl1* plants were self-fertilized we observed an increase in the proportion of kernels with severe (lethal) developmental abnormalities, suggesting that paternally contributed *Bsl1* also has a role in kernel development. Because paternally contributed *Bsl1* alone was not able to rescue defects caused by maternal *Bsl1* deficiency, it is possible that the paternal contribution either acts to reinforce the maternal gametophytic function or performs another role during seed development.

The maternal effect of *bsl1* is unique in that the mutation specifically causes detrimental effects on BETL development. Because the BETL is the primary site for the transfer of solutes from the mother plant to the seed (Thompson *et al.* 2001), an impaired BETL would produce significant changes to nutrient influx, resulting in pleiotropic effects on seed development. This phenomenon has been observed in other zygotic mutants possessing a defective BETL (Cheng *et al.* 1996; Maitz *et al.* 2000; Costa *et al.* 2003) and in maternal-effect mutants with defective connecting maternal sporophytic tissue (Felker *et al.* 1985). Moreover, an impaired BETL would certainly account for the slower growth and delayed programmed cell death in *bsl1* kernels when compared to wild-type siblings (data not shown). Similar to *reduced grain filling1* mutants in maize (Maitz *et al.* 2000), expression of BETL-specific genes was downregulated in *bsl1* mutants, although BETL-specific transcripts were often confined to discrete portions of the adgerminal and abgerminal basal endosperm. Although we are currently uncertain of the direct effects of the *bsl1* mutation on embryo development, it is likely that the retarded growth of *bsl1*/+ embryos is caused by the inability of compromised *bsl1/bsl1*/+ endosperms to support their growth. This has been shown also to be the

case for most zygotic *defective kernel* mutants by using chromosomal translocations to produce nonconcordant seeds with mutant endosperms and wild-type embryos (Neuffer and Sheridan 1980; Chang and Neuffer 1994).

BETL development is under maternal gametophytic control but is also regulated biparentally after fertilization: It is currently held that cell fate specification of the maize BETL occurs during early syncytial endosperm development (Costa *et al.* 2003). This is most likely the case in other cereals and thus far a growing number of future transfer cell-specific transcripts that are localized in a polar fashion within barley and wheat syncytial endosperms have been identified (Doan *et al.* 1996; Drea *et al.* 2005). Interestingly, we found irregular distributions of basal-specific endosperm transcripts in syncytia segregating the *bsl1* mutation, yet we did not detect any morphological abnormalities in these endosperms. In addition, irregular localization of BETL-specific transcripts was observed in *bsl1* mutant cellular endosperms, which also possessed aberrant BETL tissue. These findings therefore indicate that BSL1 is necessary for correct BETL patterning.

Studies on interploidy crosses in maize have shown that the transfer tissue is particularly sensitive to alterations in the 2 m:1 p parental genomic balance in the endosperm (Charlton *et al.* 1995; Hueros *et al.* 1999b; Gutierrez-Marcos *et al.* 2003), thus reflecting the antagonistic influences exerted by maternal and paternal genomes following fertilization. When we pollinated *bsl1*/+ plants with pollen from tetraploid plants, we found that the introduction of an extra paternal *Bsl1* wild-type allele was still not sufficient to compensate for the BETL abnormalities caused by maternal transmission of *bsl1*. Although this finding may be indicative of *BSL1* function not being dependent on dosage, the issue remains difficult to resolve since tetraploidy *per se* causes patterning defects in the endosperm. Notably, we found more severe defects in the BETL tissue of *bsl1*/*bsl1*/+/+ tetraploid endosperms than in either *bsl1*/*bsl1*/+ triploid endosperms or +/+/+/+ tetraploid endosperms. Moreover, these abnormalities ranged in severity, with the frequency of severely defective *bsl1*/*bsl1*/+/+ endosperms (18.6%) roughly correlating with the percentage of severely affected central cells in *bsl1*/+ plants (*i.e.*, 17.6%, as indicated by displaced polar nuclei). This correlation favors the idea that maternal gametophytic contribution of *Bsl1*, rather than the number of functional *Bsl1* alleles in the endosperm, is important for kernel phenotype. Moreover, these data indicate that the prefertilization gametophytic effects of *bsl1* lead directly to the formation of a more aberrant BETL. On this basis, it appears that intrinsic information for BETL patterning is present in the central cell prior to fertilization (see proposed model, Figure 7). Our findings thus contribute significantly to the model currently held for BETL development, which is believed

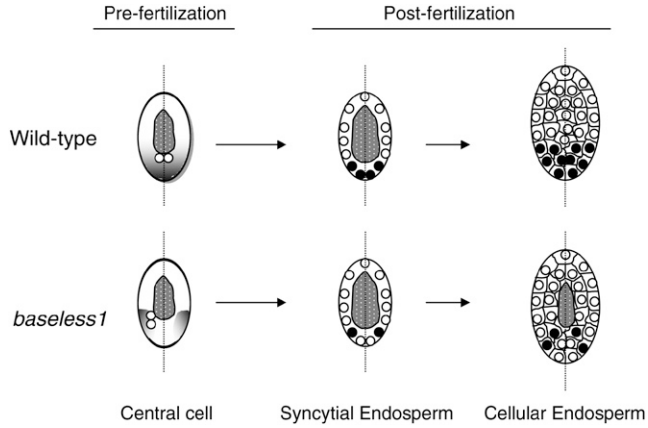

Figure 7.—Proposed model for the maternal regulation of BETL development in wild type and *bsl1*. In wild type, maternal factor(s) required for correct BETL patterning (shading) are putatively distributed along the proximal–distal axis (dashed line) of the central cell before fertilization. Mutation in *bsl1* affects development of the central cell, as indicated by the displacement of the polar nuclei (open circles) away from the central longitudinal (proximal–distal) axis. We propose that these maternal factor(s) are also displaced in *bsl1* central cells, thus altering BETL patterning. Following fertilization in wild type, BETL specification of basal nuclei (solid circles) takes place in the syncytial endosperm, with subsequent daughter nuclei and BETL cells developing in a lineage-dependent fashion (Costa *et al.* 2003). Despite fertilization of *bsl1*/+ plants with wild-type pollen, BETL specification occurs abnormally in *bsl1* syncytial endosperms, such that some basal nuclei remain undifferentiated. This leads directly to decreased BETL cell distribution and to subsequent poor nutrient uptake. As a consequence, cellularization is delayed and is indicated by the prolonged presence of the central vacuole (stippled area) in mutant endosperms, at a time when sibling wild-type endosperms are fully cellular. The future germinal side of the kernel is oriented to the right.

to occur in an irreversible lineage-dependent manner in response to a combination of developmental cues from the maternal sporophyte and from within the endosperm (Costa *et al.* 2003). These data support the proposed model (Birchler 1993) that regulation of maize endosperm development resembles that of the Drosophila blastoderm. Early patterning of the maize endosperm transfer tissue is under strong maternal control and hence resembles embryonic patterning events in Drosophila, which is achieved before fertilization via the asymmetric localization of maternal determinants within a common cytoplasm (Johnstone and Lasko 2001; reviewed in Ephrussi and St. Johnston 2004). Certainly, molecular data in Arabidopsis and maize show that there is a significant contribution of maternal products to endosperm, as well as embryo (Vielle-Calzada *et al.* 2000; Grimanelli *et al.* 2005). In addition, current genetic data highlight a large number of female gametophytic mutants that exhibit only postfertilization defects (Pagnussat *et al.* 2005). Our data further support the notion that maternally contributed factors interact with information from within the

endosperm (Birchler 1993), in this case to direct BETL patterning and development.

Analysis of *bsl1* central cells and syncytial endosperms further suggests that polarity is perturbed in both structures. We therefore favor a hypothesis in which BSL1 is required for the establishment and/or maintenance of polarity in these reproductive structures. As such, mutation in *bsl1* might affect temporal and/or spatial distribution of BETL factor(s) within the common cytoplasm of the central cell and syncytial endosperm, until endosperm cellularization takes place (see proposed model, Figure 7). However, it remains unknown how the original asymmetry is generated.

In summary, through the study of *bsl1* we have found that correct BETL patterning is predetermined in the central cell before fertilization. This information is most likely maintained within the syncytial endosperm, during which time BETL development becomes finely regulated by both maternally and paternally contributed factors.

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